Liposome Immunoassay for Rapid Identification of Group A Streptococci Directly from Throat Swabs

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The Q Test Strep (Becton Dickinson and Co., Franklin Lakes, N.J.) is a new solid-phase liposome immunoassay for the rapid diagnosis of group A beta-hemolytic streptococcal pharyngitis. Compared with blood agar plate cultures, the Q Test Strep had a sensitivity of 91%, a specificity of 83%, a positive predictive value of 88%, and a negative predictive value of 87%. Liposome technology can be used to facilitate the rapid diagnosis of group A beta-hemolytic streptococcal pharyngitis.

Antigen detection tests for the rapid diagnosis of group A beta-hemolytic streptococcal (GABHS) pharyngitis directly from throat swabs have been commercially available for approximately 6 years. The first group of these tests were primarily latex agglutination and coagglutination procedures. They were very specific but lacked sufficient sensitivity and had endpoints that were difficult to interpret (3). The next group of tests were the solid-phase enzyme immunoassays, which had better sensitivities than the earlier procedures and clearer endpoints (2, 7). The latest technological advancement in this area has been the introduction of solid-phase liposome immunoassays. This procedure is similar to the solid-phase enzyme immunoassay, but rather than using anti-group A streptococcal antibodies conjugated to an enzyme, it uses anti-group A streptococcal antibodies conjugated to a liposome (artificial phospholipid) containing a dye (rhodamine sulfate). The use of the liposome simplifies the procedure by eliminating the need to add a substrate for the antibody-enzyme conjugate in order to generate the signal. The signal is generated by the release of the rhodamine sulfate dye upon the antibody-antigen interaction. In addition, the use of the liposome reportedly increases the sensitivity (6). Recently, Becton Dickinson and Co., Franklin Lakes, N.J., developed a liposome immunoassay for the rapid diagnosis of GABHS pharyngitis called Q Test Strep. We compare this accuracy to that of the Q Test Strep with the conventional blood agar plate culture.

From winter 1988 to spring 1989, 228 consecutive patients seen in a private pediatric office (M.F.R.) with clinical findings suggestive of GABHS pharyngitis were enrolled in the investigation after informed consent had been obtained. Throat swabs were obtained by simultaneously rubbing two sterile, rayon-tipped swabs over the posterior pharynx and both tonsils (or tonsillar fossae); the first swab was a Culturette (Marion Scientific, Div. Marion Laboratories, Inc., Kansas City, Mo.) and the second swab was included in the Q Test Strep package. The first swab was immediately streaked onto a blood agar plate (Trypticase soy agar with 5% sheep blood; BBL Microbiology Systems, Cockeysville, Md.), the agar was stabbed in several areas, and a bacitracin disk (Taxo A Disc; BBL) was placed on the primary inoculum. After overnight incubation in room air at 37°C, the plates were examined for the presence of beta-hemolytic streptococci, and beta-hemolytic streptococci susceptible to bacitracin were presumptively identified as group A. If no beta-hemolytic streptococci were present after overnight incubation, the plate was reincubated for an additional 24 h. The plates were then transported to the Streptococcal Research Laboratory at the University of Connecticut Health Center for final interpretation. Beta-hemolytic streptococci were quantified as follows: 1+ culture, <10 colonies of beta-hemolytic streptococci per plate; 2+ culture, ≥10 but <50 colonies; 3+ culture, ≥50 colonies but not a pure culture; 4+ culture, ≥50 colonies in pure culture. All beta-hemolytic streptococci were grouped as A, B, C, D, F, G, or "other," using the Streptex test (Wellcome Reagents, Dartford, England).

Immediately after being obtained, the second swab was used to perform the Q Test Strep according to the instructions of the manufacturer. Briefly, the swab was placed in a plastic tube to which 3 drops of 2 M sodium nitrite and 3 drops of 2 M acetic acid had been added. After a 1-min incubation at room temperature, 5 drops of buffered 0.66 N sodium hydroxide were added and the entire contents of the tube were transferred onto a coated membrane fixed in a plastic case. After the fluid had been absorbed, 3 drops of antibody-coated (rabbit anti-group A streptococci) liposomes were added, followed by 3 drops of a buffered wash solution. A positive test result was the presence of a dark pink triangle in the center of the membrane, while a negative test result was the presence of a dark pink negative sign (−) on a white background, with no visible triangle.

All Q Test Strep tests were performed by the same nurse, who had been trained in the performance of this procedure prior to the initiation of the investigation and who was unaware of the culture results at the time the tests were performed.

Of the 228 patients from whom throat cultures were obtained, GABHS were isolated from 135 (59%). The Q Test Strep yielded positive results in 123 of the 135 patients with positive throat cultures and negative results in 77 of the 93 patients with negative throat cultures (Table 1). The Q Test Strep, therefore, had a sensitivity of 91%, a specificity of 83%, a positive predictive value of 88%, and a negative predictive value of 87% compared with blood agar cultures. Of the 12 patients with false-negative Q Test Strep results, 8 (67%) had 1+ throat cultures, while only 12% of the all the patients with positive throat cultures had 1+ cultures. Of the 16 patients with false-positive Q Test Strep results, 9 (56%) had group G beta-hemolytic streptococci (confirmed by Streptex test) and 7 had no beta-hemolytic streptococci on their throat cultures. There was a total of 14 patients with

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non-GABHS on their throat cultures: 13 patients with group G streptococci (9 with positive Q Test Strep results) and 1 patient with group B streptococci (negative Q Test Strep result). Of the nine isolates of group G streptococci from patients with false-positive Q Test Strep results, eight had been stored at \(-70^{\circ}\mathrm{C}\) and were available for later testing. When the Q Test Strep test was performed on pure cultures of these eight isolates, two produced positive results and six produced negative results.

In the only previous evaluation of a liposome immunoassay for the rapid diagnosis of GABHS pharyngitis, Huck and co-workers (5) examined a liposome immunoassay produced by Becton Dickinson (Directigen 1-2-3 Group A Strep Test) but formulated and manufactured by a different method than the Q Test Strep. They found that the Directigen 1-2-3 Group A Strep Test had a sensitivity of only 65% and a specificity of 85% compared with blood agar cultures. While the specificity of the Q Test Strep in this investigation (83%) was comparable to that of the Directigen 1-2-3 test, the sensitivity of the Q Test Strep (91%) was considerably higher.

There are several possible reasons for this discrepancy. One obviously relates to the inherent differences between these two liposome immunoassays. In addition, in the earlier report, the throat cultures were obtained by 21 different physicians and the liposome immunoassay was performed by nine different medical assistants, while in this study all of the throat cultures were obtained by the same physician and all of the liposome immunoassays were performed by the same nurse. Therefore, the greater sensitivity observed in this study might be attributable to greater consistency in the ways in which the throat swabs were obtained and the liposome immunoassay was performed. Furthermore, in the earlier study, the standard against which the liposome immunoassay was measured was a positive culture on either blood agar or selective agar plates incubated in 5% carbon dioxide, while in this study the standard was a positive culture on a blood agar plate incubated in room air. The lower sensitivity of the liposome immunoassay in the earlier study might, therefore, be due to the greater sensitivity of the selective media and the 5% carbon dioxide atmosphere used in that investigation. However, there is still a great deal of debate about the optimal culture media and atmosphere of incubation for a throat culture (4, 4a). Several investigators have found that incubation in 5% carbon dioxide does not significantly increase the yield of GABHS compared with incubation in room air and that use of selective agar plates does not significantly increase the yield of GABHS compared with blood agar plates (4, 4a). In addition, the use of both blood agar and selective agar plates by Huck et al. (5) increased the yield of GABHS by only 4% compared with the use of blood agar plates alone.

As in the study by Huck et al. (5), we found that the sensitivity of the liposome immunoassay increased with increasing degrees of positivity of the throat culture and that the liposome immunoassays were positive for a considerable number of non-GABHS. As the role of non-GABHS in acute pharyngitis is still controversial (1), the clinical implication of these false-positive results remains to be determined. However, in contrast to that earlier study (5), we did not find a large number of weakly positive liposome immunoassays that were difficult to interpret.

There are several possible explanations for the apparent discrepancies between some of the Q Test Strep results from throat swabs yielding group G streptococci and from pure cultures of the same group G streptococci. While we were careful to group several different colonies of beta-hemolytic streptococci from each throat culture plate, it is possible that some of the throat cultures contained, in addition to the group G streptococci, a small number of group A streptococci that went undetected. It is also possible that the freezing and thawing of some of the group G streptococci altered their antigenic properties. Finally, it is possible that there were specific physical properties of the throat culture compared with the pure culture that contributed to the false-positive Q Test Strep results produced by the group G streptococci.

In conclusion, we found the Q Test Strep to be a simple, rapid (approximately 3-min), and easy to interpret diagnostic test, the accuracy and predictive value of which compared favorably with those of a blood agar plate culture. Liposome technology can be used to facilitate the rapid diagnosis of streptococcal pharyngitis.

**TABLE 1. Results of Q Test Strep compared with blood agar culture**

<table>
<thead>
<tr>
<th>Q Test Strep result</th>
<th>Positive</th>
<th>Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>123</td>
<td>16</td>
</tr>
<tr>
<td>Negative</td>
<td>12</td>
<td>77</td>
</tr>
</tbody>
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**LITERATURE CITED**